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(54) Title: A METHOD FOR DETECTING ALTERATIONS IN A RENAL CARCINOMA-ASSOCIATED GENE

(57) Abstract

A method for detecting the predisposition to develop a malignant disease exhibiting a renal carcinoma-associated gene alteration. A method for detecting a renal carcinoma-associated gene alteration in renal carcinoma, small-cell lung carcinoma, non-small-cell lung carcinoma, and cervical carcinoma. The method includes hybridizing a renal carcinoma-associated gene probe to DNA extracted from target tissue and then determining the binding of the probe to the DNA. The target tissue can be tumor or non-tumor tissue and from a fetus to an adult. Further there are cDNA clones and resulting partial cDNA sequences hybridizable to the renal carcinoma-associated genes.

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A METHOD FOR DETECTING ALTERATIONS IN A RENAL CARCINOMA-ASSOCIATED GENE

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FIELD OF THE INVENTION

The present invention relates to detecting the predisposition for malignant diseases exhibiting renal carcinoma-associated gene alteration. The invention also relates to renal carcinoma-associated gene probes and their use in detecting tumors containing a renal carcinoma-associated gene alteration.

BACKGROUND OF THE INVENTION

In malignant diseases, recurring chromosome translocations are important because one or more genes located at, or adjacent to, the site of rearrangement can be altered in a quantitative or qualitative manner and thereby, lead to the development of cancer. In a

hereditary renal cancer family with the 3;8 translocation, there was complete concordance between having an elevated risk of developing cancer and the presence of the translocation (Cohen, et al., *N. Engl. J. Med.* **301**:592-595 (1979). In other words, family members who did not inherit the translocation were not at increased risk for the development of renal cancer, while those with the translocation had a predicted incidence of 79% by age 59. The family also appears to have an increase of thyroid cancer although the number of cases is less.

The involved gene is suspected to be a tumor suppressor gene because deletions of the short arm of chromosome 3 (3p) and loss of heterozygosity involving this particular segment of 3p have been regularly observed in a variety of cancers. In renal carcinoma, 3p involvement approaches 90% in the clear cell histologic variety (Kovacs, et al., *Proc. Natl. Acad. Sci. U. S. A.* **85**:1571-1575 (1988); Zbar, et al., *Nature* **327**:721-724 (1987); Ogawa, et al., *Cancer Res.* **51**:949-953 (1991); and van der Hout, et al., *Int. J. Cancer* **53**:353-357 (1993)), while in small-cell lung cancer the loss is universal (Naylor, et al., *Nature* **329**:451-454 (1987); and Yamakawa, et al., *Oncogene*. **8**:327-330 (1993)). In non-small cell lung cancer, which includes squamous, adeno, and large cell histologies, 3p loss occurs in up to 75%

of cases (Kok, et al., *Nature* 330:578-581 (1987) and Rabbitts, et al., *Genes. Chromosom. Cancer* 1:95-105 (1989)). Additionally, 3p loss has been reported in 90-100% of cervical cancers (Yokota, et al., *Cancer Res.* 49:3598-3601 (1989); Jones, et al., *Oncogene.* 7:1631-1634 (1992)) and other frequent 3p losses have been described in thyroid, head and neck, breast and testicular cancers (Herrmann, et al., *J. Clin. Invest.* 88:1596-1604 (1991); Latif, et al., *Cancer Res.* 52:1451-1456 (1992); Devilee, et al., *Genomics.* 5:554-560 (1989); Ali, et al., *J. Natl. Cancer Inst.* 81:1815-1820 (1989); and Lothe, et al., *Genomics.* 5:134-138 (1989)). In most cases, the amount of chromosome 3 material lost has been extensive. At the present time, the implication of function is by association only and lacks direct scientific proof. An alternative scenario might be that the translocation results in the activation of a gene at the breakpoint. This would implicate the gene as protooncogene in contrast to a tumor suppressor gene. Other possibilities may exist. The true function of the gene or genes await further research.

However, even without knowledge of the exact function of a gene one can envisage present or potential uses derived from knowledge of either the complete DNA sequence or partial DNA sequences of the gene. The DNA clones might be very useful diagnostic markers for kidney

cancer or other cancers such as lung and cervix. It is possible that rearrangements may be detected in early cancers. Demonstrating specific genetic changes in an early cancer or pre-malignant lesion should add important objective data to what is now a very subjective interpretation by pathologists. If the gene turns out to be a suppressor gene, then one can easily envisage replacement therapy. If activation of the gene occurs in these cancers, then antisense therapies would seem to be a viable approach.

SUMMARY OF THE INVENTION

The invention concerns a method for detecting the predisposition to develop a malignant disease exhibiting a renal carcinoma-associated gene alteration. The invention also concerns a method for detecting tumors with a renal carcinoma-associated gene alteration. In addition, the invention concerns the development of cDNA clones and probes. In accordance with one aspect of the present invention, there is provided a method for detecting the predisposition to develop a malignant disease exhibiting a renal carcinoma-associated gene alteration, comprising the steps of hybridizing a renal carcinoma-associated probe to DNA extracted from a target tissue, and determining the binding of the probe to the DNA. The target tissue is non-tumor tissue and can be fetal tissue, as well as, post-natal to adult tissue.

In other embodiments the method can be used for detecting tumors containing a renal carcinoma-associated gene alteration. For example, this method can be used to detect the alteration in renal carcinoma, small-cell lung carcinoma, non-small-cell lung carcinoma, and cervical carcinoma.

Another embodiment includes cDNA clones as well as partial cDNA sequences of the clone that are

hybridizable to the renal carcinoma-associated genes. One example is the K7 cDNA clone up to about 1.7 kb and its partial cDNA sequences, including partial cDNA sequences comprising 15 or more bases, which are all hybridizable to the renal carcinoma-associated gene 1. Other examples are the K203 cDNA clone up to about 1.3 kb, the K206 cDNA clone up to about 2.0 kb, and the K213 cDNA clone up to about 1.5 kb and their partial cDNA sequences, including partial cDNA sequences comprising 15 or more bases, which are all hybridizable to the renal carcinoma-associated gene 2.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 and 2 are partial cDNA sequences of the K7 cDNA clone.

Figs. 3, 4, 5, and 6 are partial cDNA sequences of the K206 cDNA clone.

Figs. 7 and 8 are partial cDNA sequences of the K213 cDNA clone.

Fig. 9 illustrates the location of markers surrounding the t(3;8) breakpoint. A diagram of 3p is shown along with the cytogenetic locations of four breakpoints defining the t(3;8) region. The A5-4 breakpoint is telomeric to the t(3;8) breakpoint and separated from it by approximately 500 kb. The cloned DNA markers mapped into each interval are listed along with R7K145, the only marker to map between A5-4 and t(3;8).

Figs. 10A, 10B and 10C represent the physical map of the t(3;8) breakpoint region. In Fig. 10A, the YAC contig isolated using R7K145 is shown along with the locations of all cleavage sites for *Mlu*I (M) and some for *Xho*I (X). Note that these sites have been determined on unmethylated DNA. The positions of both the t(3;8) and

aphidicolin-induced A5-4 breakpoints are indicated by the vertical shaded lines while the orientation of the contig along chromosome arm 3p is designated centromere to telomere. (R) and (L) indicate the left (CEN, TRP1) and right (URA3) ends of these pYAC4 derived YACs. The 189B12 YAC is known to be chimeric (indicated by horizontal shaded line) but the extent of the chromosome 3 portion is unknown. YAC sizes are indicated in kilobases. In Fig. 10B, a lambda phage contig situated between the two indicated *Mlu*I sites is shown. Clones 1023 and 3068 cross the t(3;8) breakpoint. Clones 2071, 4004 and 3111 are detected by the right end of YAC 74B2. In Fig. 10C, the positions of *Eco*RI cleavage sites in genomic DNA are shown for the region immediately surrounding the t(3;8) breakpoint. The shaded boxes indicate the positions of the renal carcinoma-associated gene 1 (RCA-1) and the renal carcinoma-associated gene 2 (RCA-2).

Fig. 11 shows that R7K145 YAC contig crosses the A5-4 breakpoint and the identification of the t(3;8) breakpoint by pulsed-field gel analysis. (A and B) End probes developed from the YAC clones shown in Fig. 2A were used as hybridization probes against the A5-4 and A5-5 hybrid DNAs and controls. *Eco*RI digested DNA samples included normal human (lane a), A5-4 (lane b), A5-5 (lane c) and UCTP2A3 (lane d). Hybridization with

65E7(L) is shown in panel 1 while 74B2(L) is shown in panel 2. The 65E7(L) probe is present in A5-4 but missing in A5-5 while the 74B2(L) probe is missing from both. This establishes the orientation of the YAC contig as drawn in Fig. 2A. (C) *Mlu*I digested DNA samples are from a normal human lymphoblastoid cell line (lane a) and a lymphoblastoid cell line containing the 3;8 translocation (lane b). Following separation on a pulsed-field gel, the resulting filter was hybridized with probe 65E7(R). The normal DNA fragment of 550 kb was reduced to 250 kb in the 3;8 translocation cells.

Fig. 12 shows the detection of rearranged DNA fragments at the t(3;8) breakpoint using both genomic and cDNA probes. In A, DNA samples were digested with *Xba*I. DNA samples included lane a (TL12-8), lane b (UCTP2A3), lane c (3;8/4-1). Hybridization with lambda clone 1023 revealed rearranged bands, as discussed in the text. In B and C, DNA samples were digested with *Eco*RI and hybridized with lambda clone 1023 (panel 2) or the cDNA clone K7 from the HRC1 locus (C). DNA samples are normal human (lane a), TL12-8 (lane b), UCTP2A3 (lane c) and 3;8/4-1 (lane d). Molecular weight markers are indicated.

Fig. 13 represents a schematic of genomic and cDNA clones from the region of the 3;8 translocation breakpoint.

DETAILED DESCRIPTION OF THE INVENTION

The term gene alteration as used herein refers to large and small deletions to the gene which can lead to loss of heterozygosity, point mutations to the gene, and rearrangements from chromosome translocations.

One embodiment of the present invention is a method of detecting the predisposition to developing a malignant disease exhibiting a renal carcinoma-associated gene alteration, comprising the steps of hybridizing a renal carcinoma-associated gene probe to DNA extracted from target and determining the binding of the probe to the DNA. The binding can be measured by a variety of methods, including determining the amount of probe which hybridizes, determining structural changes, restriction fragment length polymorphisms, and amplifying bound probe by the PCR method and measuring the product.

In one preferred embodiment, the probe is labelled and the hybridization is measured by determining the amount of label in the hybridized molecule. One skilled in the art will readily recognize that a variety of molecular biological methods are available to measure the hybridization itself or the product of the hybridization.

A variety of tumors have been shown to have an alteration in the renal carcinoma-associated genes. This has seen in tumor disorders such as renal carcinoma, small-cell lung carcinoma, non-small-cell lung carcinoma, and cervical carcinoma. The tumors containing the renal carcinoma-associated gene alteration can be detected by a method comprising the steps of hybridizing a renal carcinoma-associated gene probe to DNA extracted from a target tissue, and determining the binding of the probe to the DNA. For example, in renal carcinoma the tumor itself contains an altered renal carcinoma-associated gene in both chromosomes, whereas the constitutional cells may only have one renal carcinoma-associated gene alteration. Thus, it is clear that the availability of a renal carcinoma-associated gene probe provides the ability to detect individuals who are predisposed to forming a renal carcinoma tumor.

The target tissue in the sample which is obtained from individuals to be detected and from which the DNA is extracted. For example, the tissues can be tumor, blood, fibroblasts, skin or other normal tissues, and can be taken from fetal to adult tissue.

Another embodiment includes a method of detecting chromosomal DNA encoding for at least a portion of the renal carcinoma-associated gene, comprising,

denaturing total chromosomal DNA to obtain a single-stranded DNA; contacting the single-stranded DNA with a renal carcinoma-associated gene probe which can involve *in situ* hybridization; and identifying the hybridized DNA-probe to detect chromosomal DNA containing at least a portion of the human renal carcinoma-associated gene exhibiting an alteration.

One skilled in the art will readily recognize that a variety of labelled markers can be used with any of the renal carcinoma-associated gene probes. These markers can be radioactive, fluorescent or enzymatic. Furthermore, one skilled in the art will also recognize that other methods not employing a labelled probe can be used to determine the hybridization. Southern blotting, fluorescence *in situ* hybridization (Lichter and Cremer, *Human Cytogenetics Vol. I, A Practical Approach* 2nd ed. (ed. Rooney and Czepulkowski), pp. 157-192, IRL Press, Oxford)), single-strand conformation polymorphisms (Orita, et al., *Proc. Natl. Acad. Sci. U. S. A.* 86:2766-2770 (1989)) with PCR amplification, and sequence analysis involving comparison of genes are examples of methods that can be used for detecting hybridization.

Probes may be DNA or RNA. Probes may be generated by direct oligonucleotide synthesis, for example, using an Applied Biosystems (Foster City, CA) DNA synthesizer

(model 380b). Probes may be directly labelled with any detectable label known in the art, including radioactive nuclides such as ^{32}P, ^{3}H and ^{35}S, fluorescent markers such as fluorescein, Texas Red, AMCA blue, lucifer yellow, rhodamine, and the like, or with any cyanin dye which is detectable with visible light. The probe may be labelled using methods such as PCR, random priming, end labelling, nick translation or the like. Probes may also be directly labelled by incorporating nucleotides with an activated linker arm attached, to which a fluorescent market may be added. Alternatively, probes may also be indirectly labelled by incorporating a nucleotide covalently linked to a hapten or other molecule such as biotin or digoxygenin, and performing a sandwich hybridization with a labelled antibody directed to that hapten or other molecule, or in the case of biotin, with avidin conjugated to a detectable label. Antibodies may be conjugated with a fluorescent marker, or with an enzymatic marker such as alkaline phosphatase or horseradish peroxidase to render them detectable.

In situ hybridization analysis can be conducted using the probes which are determined to be positive for DNA from a renal carcinoma-associated gene. *In situ* analysis is preferably done using fluorescence. Cell samples to be used for diagnosis by the present invention can be obtained from individuals with the clinical

diagnosis of a carcinoma. If it is determined that the patient has a renal carcinoma-associated gene alteration, the patient's parents will be studied to rule out an inherited renal carcinoma-associated gene alteration.

Fetal cells may also be used for the prenatal diagnosis of the carcinoma, in which case no clinical diagnosis would have been made. These cells can be obtained through amniocentesis, chorionic villus sampling, or from the maternal circulation.

Any somatic cell type may be used. In particular, cells derived from blood samples are particularly useful for the present invention. For *in situ* hybridization analysis, the cells are placed on a solid support suitable for examination under microscopy, such as a slide or coverslip, and treated by methods well known in the art to permeablize the cells so that the detectable probe can enter the cells and bind to the renal carcinoma-associated gene. Any method known in the art of rendering a probe detectable may be used.

The probe may be labelled with a detectable marker by any method known in the art. Preferred methods for labelling probes are by random priming, end labelling, PCR and nick translation, but nick translation is preferable. For nick translation, probes may be treated

with a restriction enzyme to reduce the size of the DNA, treated with DNase I, and labelled. Labelling is conducted in the presence of DNA polymerase, three unlabelled nucleotides, and a fourth nucleotide which is either directly labelled, contains a linker arm for attaching a label, or is attached to a hapten or other molecule to which a labelled binding molecule may bind. Suitable direct labels include radioactive nuclides such as ^{32}P, ^{3}H and ^{35}S, fluorescent markers such as fluorescein, Texas Red, AMCA blue, lucifer yellow, rhodamine, and the like, or cyanin dyes which are detectable with visible light. Fluorescent markers may alternatively be attached to nucleotides with activated linker arms which have been incorporated into the probe. Probes may also be indirectly labelled, by incorporating a nucleotide covalently linked to a hapten or other molecule such as biotin or digoxygenin, and performing a sandwich hybridization with a labelled antibody directed to that hapten or other molecule, or in the case of biotin, with avidin conjugated to a detectable label. Antibodies and avidin may be conjugated with a fluorescent marker, or with an enzymatic marker such as alkaline phosphatase or horseradish peroxidase to render them detectable. Conjugated avidin and antibodies are commercially available from companies such as Vector Laboratories (Burlingame, CA) and Boehringer Mannheim (Indianapolis, IN).

The enzyme can be detected through a colorimetric reaction by providing a substrate and/or a catalyst for the enzyme. In the presence of various catalysts, different colors are produced by the reaction, and these colors can be visualized to separately detect multiple probes. Any substrate and catalyst known in the art may be used. Preferred catalysts for alkaline phosphatase include 5-bromo-4-chloro-3-indolylphosphate (BCIP), nitro blue tetrazolium (NBT) and diaminobenzoate (DAB). Preferred catalysts for horseradish peroxidase include orthophenylenediamine (OPD) and 2,2'-azinobis (3-ethylbenz-thiazolinesulfonic acid) (ABTS).

Probes in *in situ* hybridization may be DNA or RNA. Probes may be generated by direct oligonucleotide synthesis, for example using an Applied Biosystems (Foster City, CA) DNA Synthesizer (model 380b).

Multiple probes spanning the renal carcinoma-associated gene may be used. These multiple probes may be overlapping, or may be positioned with the 3' end of a first probe directly adjacent to the 5' end of a second probe. Alternatively, these probes may not span the entire renal carcinoma-associated gene, but may span a sufficient sequence to detect whether a renal carcinoma-associated gene is present.

Hybridization of the detectable probes to the cells is conducted with a probe concentration of 0.1-500 ng/μl, preferably 5-250 ng/μl, and most preferably 10-120 ng/μl. The hybridization mixture will preferably contain a denaturing agent such as formamide, and non-specific human DNA, preferably derived from the placenta, which is used to block repeat sequences. The non-specific DNA is added at a concentration of 100 ng/μl - 2 μg/μl, more preferably 0.2-1 μg/μl, and most preferably 0.25-0.5 μg/μl to compete out any repetitive portions of the probe.

Hybridization is done in the presence of probes which are specific for a renal carcinoma-associated gene. Hybridization is carried out at 25-45°C, more preferably at 32-40°C, and more preferably at 37-38°C. The time required for hybridization is about 0.25-96 hours, more preferably 1-72 hours, and most preferably for 4-24 hours. Hybridization time will be varied based on probe concentration and hybridization solution content which may contain accelerators such as hnRNP binding protein, trialkyl ammonium salts, lactams and the like. Slides are then washed with solutions containing a denaturing agent, such as formamide, and decreasing concentrations of sodium chloride or in any solution that removes unbound and mismatched probe.

The temperature and concentration of salt will vary depending on the stringency of hybridization which is desired. For example, high stringency washes may be carried out at 42-68°C, while intermediate stringency may be in the range of 37-55°C, and low stringency may be in the range of 30-37°C. Salt concentration for a high stringency wash may be 0.5-1x SSC (3M NaCl, 0.3M Na citrate), while medium stringency may be 1X-4X, and low stringency may be 2X-6X SSC.

The detection incubation steps, if required, should preferably be carried out in a moist chamber at 23-42°C, more preferably at 25-38°C and most preferably at 37-38°C. Labelled reagents should preferably be diluted in a solution containing a blocking reagent such as bovine serum albumin, non-fat dry milk or the like. Dilutions may range from 1:10-1:10,000, more preferably 1:50-1:5000, and most preferably at 1:100-1:1000. The slides or other solid support should be washed between each incubation step to remove excess reagent.

Slides may then be mounted and analyzed by microscopy in the case of a visible detectable marker, or by exposure to autoradiographic film in the case of a radioactive marker. In the case of a fluorescent market, slides are preferably mounted in a solution which contains an antifade reagent, and analyzed using a

fluorescence microscope. Multiple nuclei may be examined for increased accuracy of diagnosis.

Screening for tandemly repeated sequence polymorphisms can be done by any method known in the art, but it is preferably conducted using PCR. Nucleotide repeat sequences include variable number tandem repeats (VNTRs), short nucleotide repeats including tri- and tetrameric repeats (Edwards et al., *Am. J. Human Genetics* 49:746-756 (1991)) and dinucleotide repeats. Common dinucleotide repeats include CA repeats which are preferable for use in the practice of the present invention. The renal carcinoma-associated gene can be screened for CA repeats by any method known in the art, including cloning into an M13 vector and directly sequencing, or by using a combination of Alu and CA or GT primers by the method of Feener et al., *Am. J. Hum. Genet.* 48:621-627 (1991). Primers are preferably 12-50 nucleotides long, more preferably 20-40 nucleotides long, and most preferably 20-30 nucleotides long. Multiplexing is a preferred method of carrying out multiple PCR reactions at the same time in a single tube.

PCR products generated using these primers can be cloned into an appropriate sequencing vector such as pBS (Bluescribe) or pBluescript (Stratagene, La Jolla, CA) and sequenced using dideoxy sequencing. Confirmation

that the tandemly repeated sequence polymorphism is found on a renal carcinoma-associated gene may be done using PCR on somatic cell hybrids. Screening for VNTRs may be done by hybridizing a total human library using consensus sequences such as those disclosed by Nakamura et al., *Science* **235**:1616-1622 (1987), and identifying clones positive for the consensus sequence which also map to a renal carcinoma-associated gene.

Sequences flanking a dinucleotide repeat region may be used as PCR primers to generate PCR products which are labelled with ^{32}P, and which contain the entire dinucleotide repeat. The polymorphism in the dinucleotide repeat can be determined by running the products on a polyacrylamide gel to resolve alleles. By comparing the length of the dinucleotide repeat among individuals, most preferably between parents and offspring, one may determine whether the critical chromosomal region was derived from one or both parents. Alternatively, in the case of VNTRs, a restriction digest of genomic DNA may be run on a gel, Southern blotted and hybridized with a VNTR probe to determine its size.

A further embodiment includes the cDNA clones and resulting partial cDNA sequences involved in the isolation of renal carcinoma-associated genes 1 and 2. The isolation of each gene was achieved by the positional

cloning of the 3p14.2 breakpoint and the isolation of cDNA clones adjacent to the site of rearrangement. The partial cDNA sequences of the K7 cDNA clone are set out in Figs. 1 and 2. The partial cDNA sequences of the K206 cDNA clone are set out in Figs. 3, 4, 5, and 6. The partial cDNA sequences of the K213 cDNA clone are set out in Figs. 7 and 8. DNA sequencing is accomplished by the deletion subcloning method as described in Henikoff, *Gene* 28:351-359 (1984). In this method deletions are made from initial clones, re-subcloned into a plasmid and new sequence information is obtained using a primer adjacent to the cloning site.

Identification of a Chromosome 3 Marker in Close Proximity to the t(3;8) Breakpoint

The development of a somatic cell hybrid mapping panel and nearly 500 regionally localized DNA probes for chromosome 3 has been previously described (Drabkin, et al., *Genomics*. 8:435-446 (1990)). In addition to hybrids containing the derivative 3 and derivative 8 chromosomes from the t(3;8) family, three additional hybrids were used to identify a subset of markers in close proximity to the translocation breakpoint. These breakpoints are shown in Fig. 9. DNA probes mapping between the 3;6 and 3;8 translocation breakpoints were tested against DNA from the A5-4 hybrid

which contains an aphidicolin induced breakpoint in 3p14. As shown schematically in Fig. 9, of 14 markers only one, R7KAC1-6#145, was retained in hybrid A5-4. This suggested that the distance between the 3;8 and A5-4 breakpoints was small.

Hybrid A5-5 allowed definition of a subset of markers immediately centromeric to the 3;8 translocation. By molecular analysis, R7KAC1-6#145 had been lost along with approximately 25% of probes previously mapped to the 3p13-p14.2 region (Drabkin, et al., *Genomics*. 8:435-446 (1990)). This defined a subset of markers adjacent to the 3;8 translocation on the centromeric side and reinforced the placement of R7KAC1-6#145.

Molecular Cloning of the Translocation Breakpoint Region

Chromosomal walking was initiated on both sides of the breakpoint using corresponding YACs isolated from the Centre d'Etudes du Polymorphisme Humain YAC library (Albertsen, et al., *Proc. Natl. Acad. Sci. U. S. A.* 87:4256-4260 (1990)). Three YACs were isolated using probe R7KAC1-6#145 and a contig was established on the basis of end-clone analysis (Fig. 10A). The left-end (TRP1) of YAC 74B2 was found to cross the aphidicolin induced breakpoint present in hybrid A5-4 (Fig. 11), thus establishing the orientation of the contig. A probe

derived from the right-end of YAC 65E7, located between the 3;8 and A5-4 breakpoints, detected an altered fragment in an *Mlu*I digest of DNA bearing the 3;8 translocation (Fig. 11, C). The differences observed between the sizes of the *Mlu*I fragments detected in YAC DNA (shown in Fig. 2A) and the human DNA (shown in Fig. 11, C) are due to methylation involving the two *Mlu*I sites on the centromeric side of the translocation breakpoint.

A subsequent walking step in the centromeric direction was performed. YAC 850A6 (Fig. 10A) was found to cross the 3;8 breakpoint by both end-clone analysis and fluorescence *in situ* hybridization. A lambda phage library was constructed from the 1.3 Mb 850A6 YAC and approximately 7 equivalents of human recombinants were identified and placed onto grids. Random human clones were mapped with respect to the 3;8 translocation breakpoint using the der(3) and der(8) somatic cell hybrids and positioned on the *Mlu*I map of the 850A6 YAC shown in Fig. 10A. This allowed localization of the breakpoint to a 185 kb *Mlu*I fragment. This fragment was gel-purified and used as a hybridization probe to identify the corresponding subset of human lambda clones. End-probes were isolated by *Eco*RI/*Sal*I double-digestions and used to establish the phage contig shown in Fig. 10B. Clones 1023 and 3068 were found to span the breakpoint.

Fig. 12, (A) shows an *XbaI* digest in which probe 1023 detects a rearrangement on the centromeric side of the breakpoint. Germline fragments of approximately 8.0, 5.0 and 3.0 kb are seen in the normal chromosome 3 hybrid (lane b). The 5.0 kb fragment is present in the der(8) hybrid (lane c), while the 3.0 kb fragment and a rearranged band of approximately 15.0 kb are present in the der(3) hybrid (lane a). In Fig. 12 (B), germline *Eco*RI bands of approximately 12.0, 7.0 and 2.5 kb were detected using probe 1023 in total human DNA (lane a) and in the somatic cell hybrid containing a normal chromosome 3 (lane c). In contrast, only the 2.5 and 7.0 kb bands were present in the der(3) containing hybrid (lane b), while an altered fragment of approximately 20 kb on the telomeric side of the translocation could be seen in the der(8) hybrid (lane d).

Isolation of a cDNA Clone Adjacent to the Translocation Breakpoint

Individual lambda clones shown in Fig. 10B were tested for both unique and evolutionarily conserved DNA sequences and used to screen an adult kidney cDNA library. Three isolates were identified from approximately 600,000 plaques using a fragment from lambda clone 4040 which is adjacent to the breakpoint on the telomeric side. The largest of these cDNA clones,

K7, contains a 1.7 kb insert with a polyA tract. On Southern hybridization, the K7 probe detects the 3;8 translocation breakpoint on *Eco*R1 digestion of DNA from the der(8) hybrid. This is shown in Fig. 12 (C). The band sizes correspond to those seen with the lambda clone, 1023, indicating that all the K7 cDNA sequences are contained within the 12.0 kb *Eco*R1 genomic fragment of the renal carcinoma-associated gene 1.

A second gene, renal carcinoma-associated gene 2, represented by cDNA clones K203 (1.3 kb), K206 (2.0 kb), and K213 (1.5 kb), was identified using the same kidney cDNA library by an evolutionarily conserved fragment from the lambda clone 1023 that crosses the 3;8 translocation breakpoint. In Fig. 13, a schematic shows which cDNA clones correspond to the genomic clones 4040 and 1023.

The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

EXAMPLE 1

Cell Lines

Somatic cell hybrids containing the derivative 3 and 8 chromosomes from the 3;8 translocation have been described (Drabkin, et al., *Proc. Natl. Acad. Sci. U. S. A.* 82:6980-6984 (1985)). Hybrid TL12-8 contains the der(3) chromosome (8qter-8q24.1::3p14.2-3qter) as its only cytogenetically identifiable human material, whereas hybrid 3;8/4-1 contains the der(8) chromosome (8pter-8q24.1::3p14.2-3pter) in the absence of the normal 3, 8 or der(3) chromosomes. Prior to DNA preparations, the hybrids were subcloned and examined cytogenetically as described (Drabkin, et al., *Proc. Natl. Acad. Sci. U. S. A.* 82:6980-6984 (1985)). Hybrid A5 contains an aphidicolin induced terminal deletion of 3p with a breakpoint at approximately 3p14 (Glover, et al., *Am. J. Hum. Genet.* 43:265-273 (1988)). This hybrid was derived from UCTP2A3 which contains an intact human chromosome 3 as its only identified human material (Drabkin, et al., *Genomics*. 8:435-446 (1990)). Routine sub-culturing of A5 cells resulted in two clones that were used in subsequent experiments. Clone A5-4 appeared identical to the original A5 hybrid. Clone A5-5 contained CHO chromosomal material spontaneously translocated onto the end of the deleted human 3p arm. No other changes to the human

chromosome 3 were cytogenetically apparent. However molecular analysis of clone A5-5 showed that some 3p material had been lost. The hybrid 3;6/UC2 retains the der(3) chromosome (6pter-6p11::3p14.3-3qter) present in a constitutional translocation associated with hereditary hematological malignancies (Markkanen, et al., *Cancer Genet. Cytogenet.* **25**:87-95 (1987)).

EXAMPLE 2

DNA Probes

The development of a chromosome 3 somatic cell hybrid mapping panel and a large series of localized probes has been described (Drabkin, et al., *Genomics*. 8:435-446 (1990)). Probe R7K145 was isolated from a partial-*Mbo*I digest genomic library constructed in EMBL3 using DNA from a radiation-reduced hybrid, R7K1-6. This hybrid was found to contain the markers MS156 and MS453 (Drabkin, et al., *Genomics*. 8:435-446 (1990)) which mapped to the general region of the 3;8 translocation. Genomic probes derived from the 850A6 YAC were isolated from partial-*Mbo*I digest libraries using two separate vectors, lambda FIX II (Stratagene, La Jolla, CA) and EMBL3cos-Not (Dr. Noreen Murray, University of Edinburgh, Scotland). The libraries were constructed using standard procedures (Sambrook, et al., *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), p. 9.4-9.59 (1989)).

EXAMPLE 3

YAC and cDNA Library Screening

YAC clones were isolated from the C.E.P.H. human YAC library prepared in the vector pYAC4 (Burke, et al., *Science* 236:806-812 (1987)) and from additions to this library containing megabase-sized inserts. YACs were isolated using conventional hybridization and PCR screening approaches (Mendez, et al., *Genomics* 10:661-665 (1991); Green and Olson, *Proc. Natl. Acad. Sci. U.S.A.* 87:1213-1217 (1990)). YACs positive for a given marker were purified by single colony isolation and then tested to confirm the presence of the marker by digestion with EcoRI and Southern blot analysis. Restriction maps of YACs were constructed using partial digests and hybridization to pYAC4 end-probes. Lambda phage sub-clones of the 850A6 YAC were used as probes to screen an adult human kidney cDNA library (Clontech, Inc., Palo Alto, CA). All Southern hybridizations were performed using a charged nylon membrane (Oncor, Inc., Gaithersburg, MD) in a buffer described by Amasino (Amasino, R. M., *Anal. Biochem.* 152:304-307 (1986)). The RNA isolation and Northern blot analysis were according to standard protocols (Sambrook, et al., *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), p. 9.4-9.59 (1989)).

EXAMPLE 4

Fluorescence *In Situ* Hybridization

The DNA clones derived from the hereditary renal carcinoma 3;8 translocation breakpoint are labeled appropriately for fluorescent *in situ* hybridization studies and hybridized to metaphase chromosomes from renal cell carcinomas or other carcinomas likely to contain deletions of the 3p14 region. The absence of hybridization signal from one or more of the chromosome 3 alleles present in the tumor sample is taken as evidence that the chromosomal region has been deleted (tumors may contain more or less than the normal two copies of each human chromosome). The finding of such deletion is, in fact, evidence for genetic instability and a marker of malignant or premalignant change as long as the individual in question does not carry an inherited or acquired deletion in his/her normal cells. The finding of a 3p14 deletion is expected to carry the same importance as detecting mutations or other alterations in other genes (oncogenes or tumor suppressor genes) known to be involved in the development of cancer.

EXAMPLE 5

Single-Strand Conformation Polymorphisms

DNA from one or more cells, or from a larger portion of a suspected cancer or premalignant lesion, is specifically amplified using the polymerase chain reaction and internally labelled with radioactive phosphorus (32-P) or other approaches (such as fluorescent dyes) that allow the subsequent detection of DNA. This DNA is denatured and electrophoresed (separated) on non-denaturing polyacrylamide gels as described in Orita, et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:2766-2770 (1989). This allows individual single-stranded DNA molecules to fold upon themselves in a manner that is a function of the primary DNA sequence of that molecule. Differences in the DNA sequence confer a change to the folding pattern and result in a migration difference between the normal molecule and one that contains an alteration in one or more nucleotides that make up the DNA. Thus, altered migration (either slower or faster than normal) indicates a change in the DNA sequence.

The DNA sequence for a given segment of DNA obtained from normal cells does not differ among cells. Alterations in the DNA sequence are a frequent event in

genes known to be altered in the development of cancer or premalignant disorder.

DEPOSIT OF STRAINS USEFUL IN PRACTICING THE INVENTION

A deposit of biologically pure cultures of the following strains was made with American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md., the accession numbers indicated was assigned after successful viability testing, and the requisite fees were paid. Access to said cultures will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. Section 1.14 and 35 U.S.C. Section 122. All restriction on availability of said cultures to the public will be irrevocably removed upon the granting of a patent based upon the application and said cultures will remain permanently available for a term of at least five years after the most recent request for the furnishing of a sample and in any case for a period of at least 30 years after the date of the deposit. Should the cultures become nonviable or be inadvertently destroyed, they will be replaced with viable cultures of the same taxonomic description.

The human genomic DNA clone, inserted into the lambda FIX, has been designated lambda 1023 and has been

given ATCC No. 75552 and deposit date August 25, 1993. The human cDNA clone, inserted into the lambda gt10, has been designated K7 and has been given the ATCC No. 75553 and deposit date August 25, 1993.

As will be apparent to those skilled in the art in which the invention is addressed, the present invention may be embodied in forms other than those specifically disclosed above without departing from the spirit or essential characteristics of the invention. The particular embodiments of the present invention described above, are, therefore, to be considered in all respects as illustrative and not restrictive. The scope of the present invention is as set forth in the appended claims rather than being limited to the examples contained in the foregoing description.

WHAT IS CLAIMED IS:

1. A method for detecting the predisposition to develop a malignant disease exhibiting a renal carcinoma-associated gene alteration, comprising the steps of:

hybridizing a renal carcinoma-associated gene probe to DNA extracted from a target tissue; and determining the binding of the probe to the DNA.

2. The method of claim 1, wherein the renal carcinoma-associated gene is renal carcinoma-associated gene 1.

3. The method of claim 1, wherein the renal carcinoma-associated gene is renal carcinoma-associated gene 2.

4. The method of claim 1, wherein the target tissue is non-tumor tissue.

5. The method of claim 2, wherein the target tissue is fetal tissue.

6. The method of claim 1, wherein the renal carcinoma-associated gene probe is labelled.

7. A method of detecting tumors containing a renal carcinoma-associated gene alteration, comprising the steps of:

hybridizing a renal carcinoma-associated gene probe to DNA extracted from a target tissue; and

determining the binding of the probe to the DNA.

8. The method of claim 7, wherein the tumors are selected from the group consisting of renal carcinoma, small-cell lung carcinoma, non-small-cell lung carcinoma, and cervical carcinoma.

9. A method of detecting chromosomal DNA encoding for at least a portion of a human renal carcinoma-associated gene exhibiting an alteration, comprising the steps of:

treating total chromosomal DNA under denaturing conditions to obtain single-stranded DNA;

contacting the single-stranded DNA so obtained with a renal carcinoma-associated gene probe; and

identifying the hybridized DNA-probe so formed to detect chromosomal DNA containing at least a portion of the human renal carcinoma-associated gene exhibiting an alteration.

10. The method of claim 9, wherein the renal carcinoma-associated gene probe hybridizes *in situ* with the single-stranded DNA.

11. A purified and isolated K7 cDNA clone of up to about 1.7 kb, wherein the clone is hybridizable to the renal carcinoma-associated gene 1.

12. A cDNA probe hybridizable to the renal carcinoma-associated gene 1, wherein the probe is a partial cDNA sequence of the cDNA clone of claim 11, selected from the group consisting of:

the partial cDNA sequences set out in Figs. 1 and 2; and

partial cDNA sequences comprising 15 or more bases.

13. A purified and isolated K206 cDNA clone of up to about 2.0 kb, wherein the clone is hybridizable to the renal carcinoma-associated gene 2.

14. A cDNA probe hybridizable to the renal carcinoma-associated gene 2, wherein the probe is a partial cDNA sequence of the cDNA clone of claim 13, selected from the group consisting of:

the partial cDNA sequences set out in Figs. 3, 4, 5, and 6; and

partial cDNA sequences comprising 15 or more bases.

15. A purified and isolated K213 cDNA clone of up to about 1.5 kb, wherein the clone is hybridizable to the renal carcinoma-associated gene 2.

16. A cDNA probe hybridizable to the renal carcinoma-associated gene 2, wherein the probe is a partial cDNA sequence of the cDNA clone of claim 15, selected from the group consisting of:

the partial cDNA sequences set out in Figs. 7 and 8; and

partial cDNA sequences comprising 15 or more bases.

17. A method of detecting a renal carcinoma-associated gene alteration, comprising the steps of:

hybridizing a cDNA clone or a partial cDNA sequence thereof of a renal carcinoma-associated gene to DNA extracted from target tissue; and

detecting the hybridization.

CGGTATTTGTGGTGAGCAGACATGGCTAAGTGCCTCTTCCTATCTAGGGAGGTTCAGGGAATTACTCAAGATCTCTTTTG
TGCCAGGGAACTAGGGAGTCAGTCATCTCATTTGCTTTCATTTCCTCATCTCTGAAATTTCCTACTATGGTATTCCTCTT
AGAACACCATTTAGAAGTGTTATGGTTTTCTGTTCTTAATCCAAATGTGACAAAATGTATTCTCCTTCTTCTCTGATAAC
AAAACCCAAATTCTGACGGGCAGCATGTGCTTAGCCTTAGATAATGAATAATGACTGGTCTAACCCAATCATGGCGATAC
TATGTTCTTTTGCCATTGATTGGTATTCAGTAGTAGGCATGTGACCTAGATCTAACCAATGAGTGGACATACACGTGGGA
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CTTTCCTGTTTTAGATGCTGTCATGTTAACGGCTTTGTGCTTAGAGCTGTACAAGCCGTCTTGTGACCATGAGGCAATAG
CGGGAAGAGGAAAGAGCCTGAGTATGAAAGACACTGGGTCCTTTATGATATTACTGAGTTGCCAAACTAACCTGGGATCC
TCTACCTCCTGTTTTCTCATAGTAATAAATATCCTGTGATTTCAGCACTATCACTTTAATTTTCTGTTGCAGGCAGCTGA
AGCATTCAGAACTCACATACTATCTTAACATCATCTTCCATGTGTTATGGGCCAAGGATATTCAAGAGTGACTACAAAAC
TACATTCATTGTCGTATCCCTGTGGGACTCCCTTAAAGTTCTAGTGTTCTGTAGTGTACTTTCCACAGCCTAGAAGGATA
TATGTTTCCCTCTCTCCAGCCCTTA

FIG. 1

CGTAACAACAAGCAAGAAAAGCAAAGAACCTTGTAAACACCCATGTTATGGAGTGTGAGAGTGTAAGCTTAAGGAGGCTT
TTATTTTGGGGAGAAGATGCATCACCTCTGTTTGAATGAGAGAAATTAAGTTGTAATTATGATGGTGTTTCAGTTAAATT
ATTTGATGTGGAAGACAAGGAGACCATTCCCAACATGTTATTTAGCAAAGAATATGGAGGAGAGGCAGAAGACTGTGATG
GAAAACGTTCACAGGCCATCAACCAAAATGGTAATGCATTCGAGGCAGATAAAGTTGCTTCTTGCTAGACAGAGGTGGGG
TGAGCCCTGACATACGTCTTCAAAATTGTTAAAGGCACAGATCTGGGGGGAAAAGGGGGTTGATTCACCAAATCTCAGAA
TAGCAGAATGGCAATAACCCTACTCCAAGGCTTCCAGTTTTCCTCAGTTTAACTCCTTTTGCATACTGCAGCCAAAGAGA
TTTTTTTTAAAATGAAGATCACTCAGACTGTGTCCTCTTCCTCTTAATCTCCCCAAAGGAAGAAAAAAACAATGATCAAT
GGCAAATGGCAGTTGCAATGAAGCAACACCAAGAACGAGCTTCGTGCTCGGGAGAGAACATTGCATATCTTCCTGTGGTT
TCCAGTGCTCTACACACTTGGGGAAACTCCGGAAGATATGCAATGTTCTCTCCCGAGCACGAAGCTCGTTCTTGGTGTTG
CTTCATTG

FIG. 2

AAATTTCAGAAAGTGAGACCTTGGGGTGGGAGATGGGGCACGCTTGGATTCGGTGTCTAACTCTAATTCAAACAACTTTT

GCCACGGAGCCATGTAGTAGCCAAATATGTTTCTAAGGCCCAAGGGACCACTCTTACAAAGATGGAAGATAGTTCACTAA

AAAGAAGACATGCTTTCTAGACTCCACAGCCACCCCAGAAGTGCCAAGATAAATAATAGGGAGCCAGAGTTTAAGCAAGG

GCCACCATCATCGCAAGTTGTCTCATCCAACTTCTCCCTTA

FIG. 3

TTTTTCTACCCATAAACATGTTAATATTCTCTATCAGTTGAAAGTCAAGTCTTGCTGACCTGTTTTTTTTTCTTTGTGTG

TTTAAATACCTCTGTATTGATTCCATTATGCATATAACAGAGCAATATTAGTTTTCTCATTTCTGAGTTCTGTGGGCATGC

ACTTACGTGCTCATACAGCTGAAAATAGTAGGTGACAGAATGAGCAGCAAGGTGAA

FIG. 4

CAGGAATTCAGAGATATGCAGTCGTTCCTTCATGTGGCTAGAACTATGACAGGTAACCTGAGATCTGTGGACAACCATAT

TTCCTATGAGAATTGAGAAGCAGAGATAGAAGGGGGCTGTATCCCTGAGTTCCTGCCCACCTTCTGGTTCCCGGCACTGG

GGGGAACTGGCTGCATCTTTGCCTCTGAGTCCTTAGACACTCTTGAGGTCCTTCTAATGATGCCCTTAAT

FIG. 5

GGAAATCCCAAGGAACCAACCTCAGATTGGTCTCTTACCTAAGGAAAGATTCTGGCAACACAACAGGAAGGATTTGGAAA

ATTTAATGAAGATTTTGAAATACCCCTAACACACTTAAATTAAAGATAATACCTATTTCACCCAAATCTCTAACCCCCTG

TGGAACAGCAT

FIG. 6

TGGATTCCACTAATGAGCACCCATCAAGTGTTTAGTAAACCTCAGATTGATGAATGATAGGTGATAGGCATACTTACCTA

TTTCTTAACCCTTTCTTTCTGTGATATTATGCATATGGTTTATTAGTCTCTATACGTCATAACAAACTATGATGAATTT

AGTGACTTAAAACAACACACATTTATTATCTGGCAGTCAGAAGCCCAATACTGTCTCACTGGGCTAAAATCGAGGATCAG

CAGGGTGTTCTACTGGAGACTGCAGAAAAACCAACTTCAAA

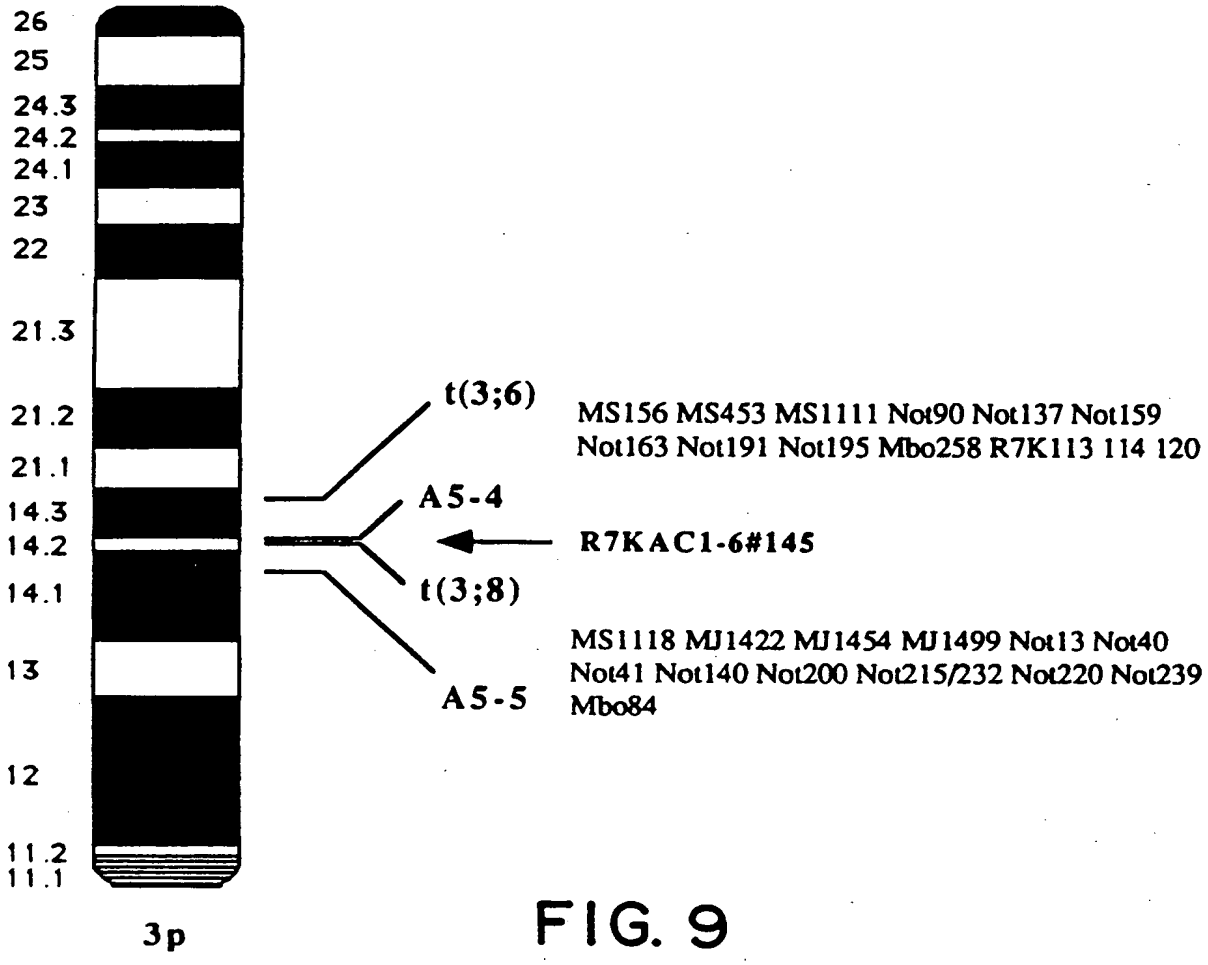
FIG. 7

GCTGGCGCCGGGTTACCGCCTGCCACCATATGAGATGTACCTTTGCTCCTCCTTCACCTTCTGCCATGATTATAGGCTTC

TCAGCCATGTGGAACTGTGAGTCATTAAACTTCTTTTTCTTTATAAATTATCCATCTCGGGTATTCTTCATAGCAGTATA

AAATGGACTAATACGTCTCAATAGGCTTGTTGTGAACCCAAATGAGACAA

FIG. 8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25
24.3
24.2
24.1
23
22
21.3
21.2
21.1
14.3
14.2
14.1
13
12
11.2
11.1
3p
t(3;6)
MS156 MS453 MS1111 Not90 Not137 Not159
Not163 Not191 Not195 Mbo258 R7K113 114 120
A5-4
R7KAC1-6#145
t(3;8)
MS1118 MJ1422 MJ1454 MJ1499 Not13 Not40
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A5-5

FIG. 9

FIG. 10A

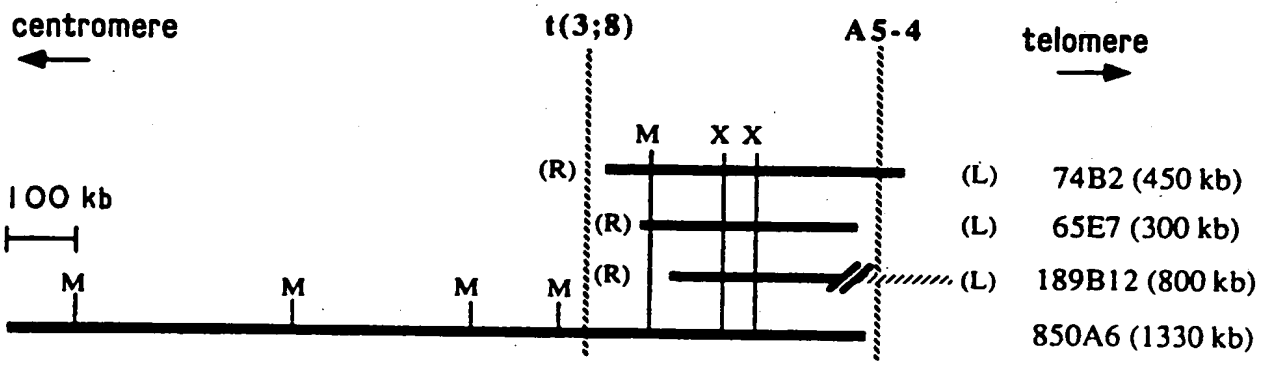
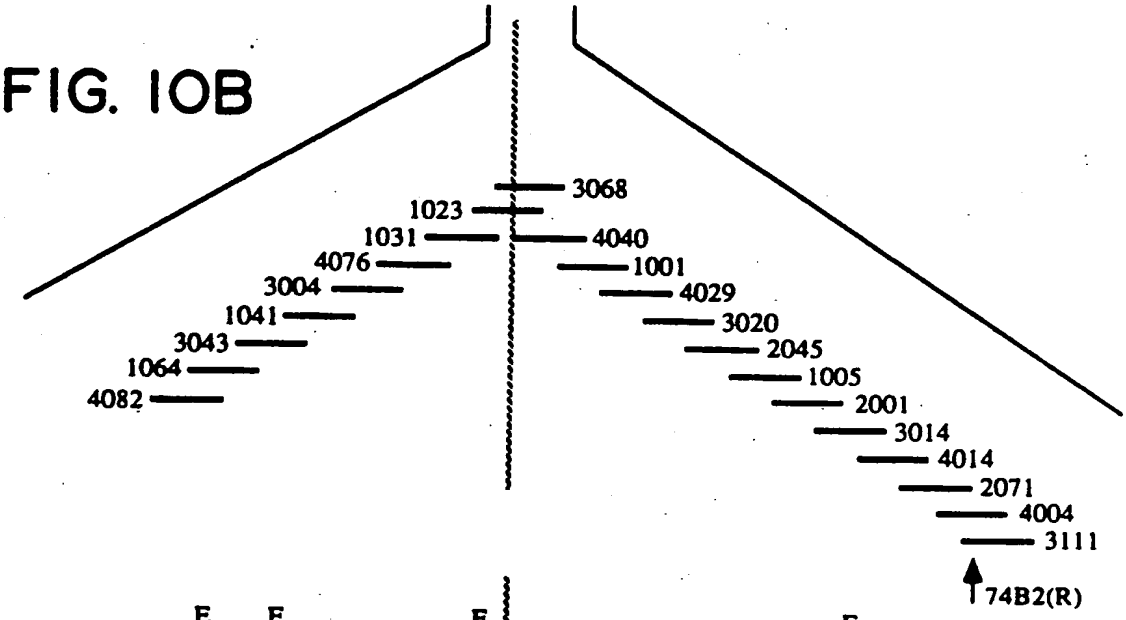


FIG. 10B



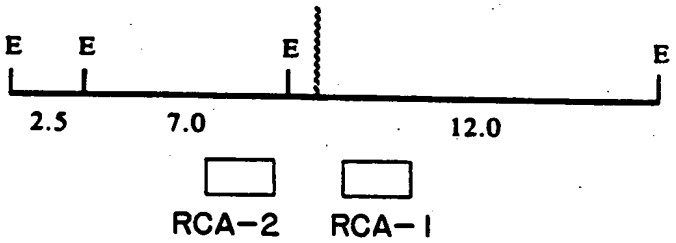


FIG. 10C

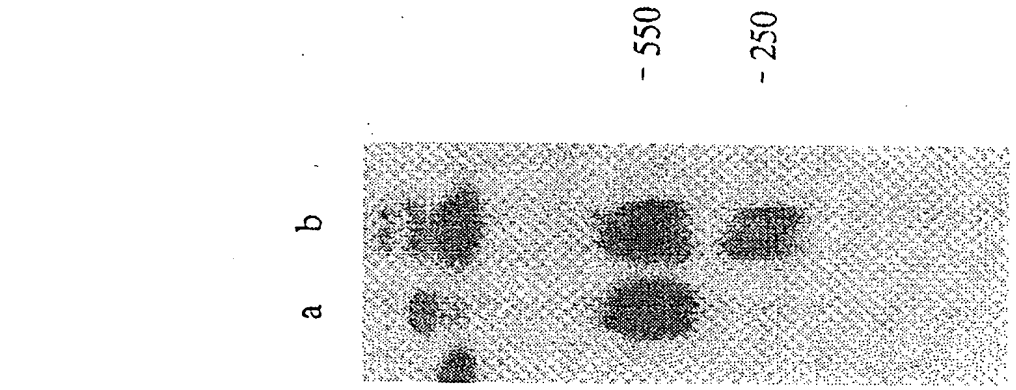


FIG. IIC

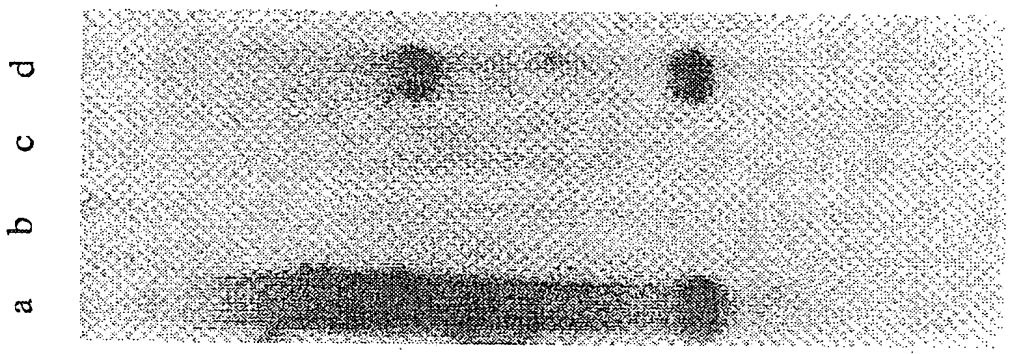


FIG. IIB

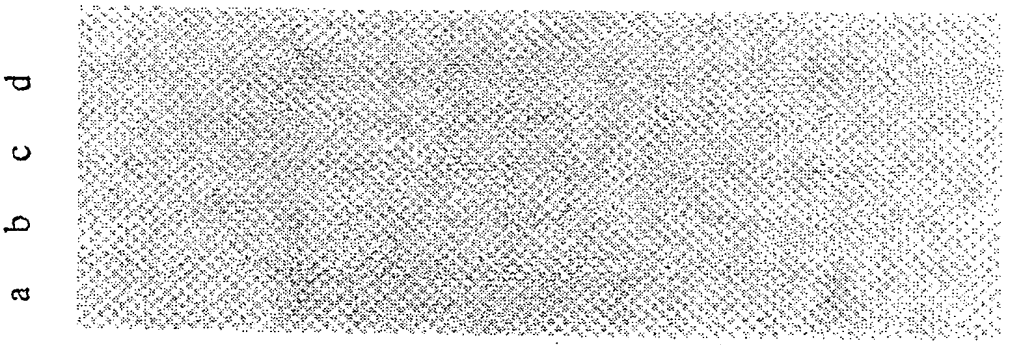


FIG. IIA

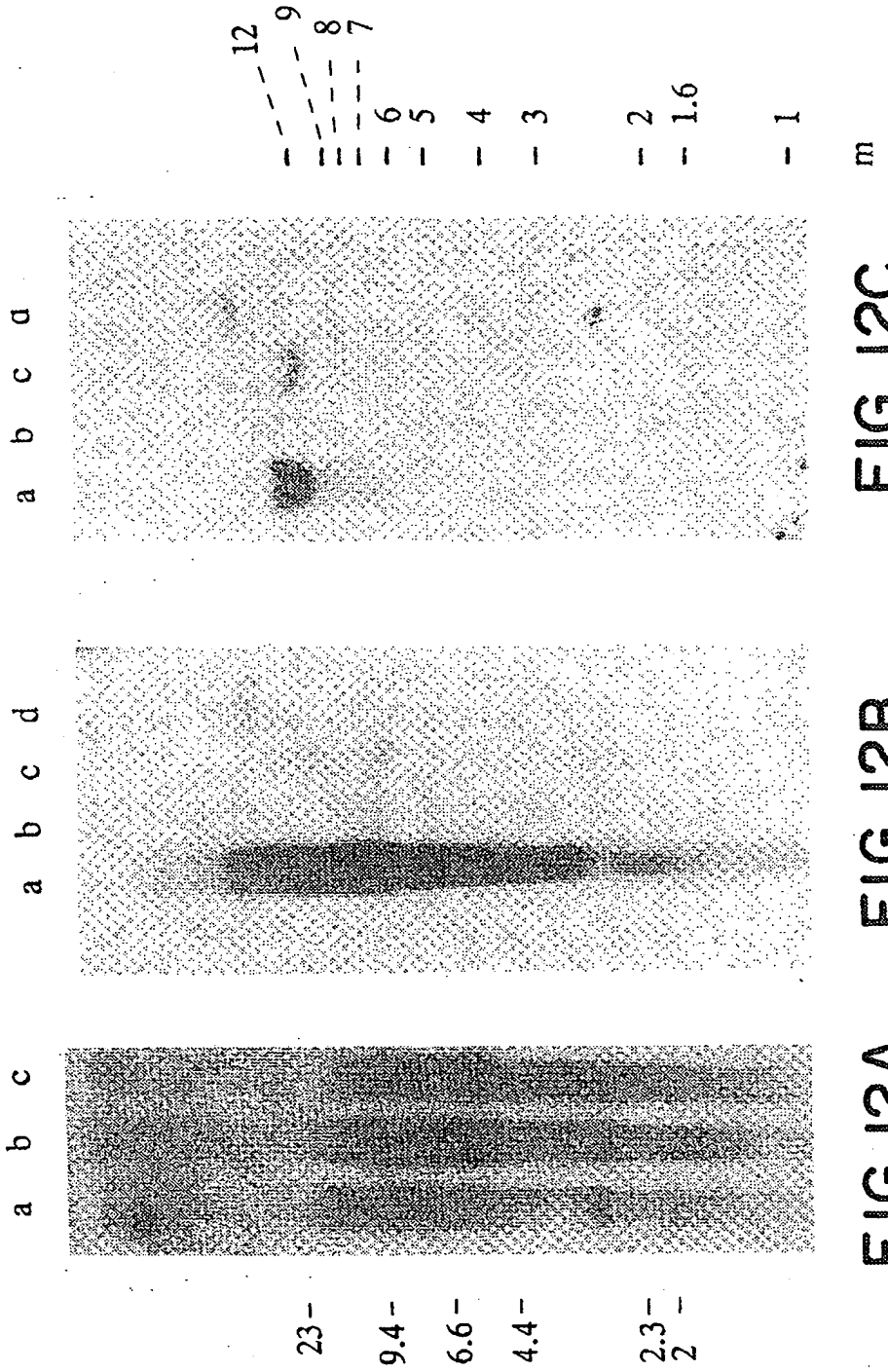
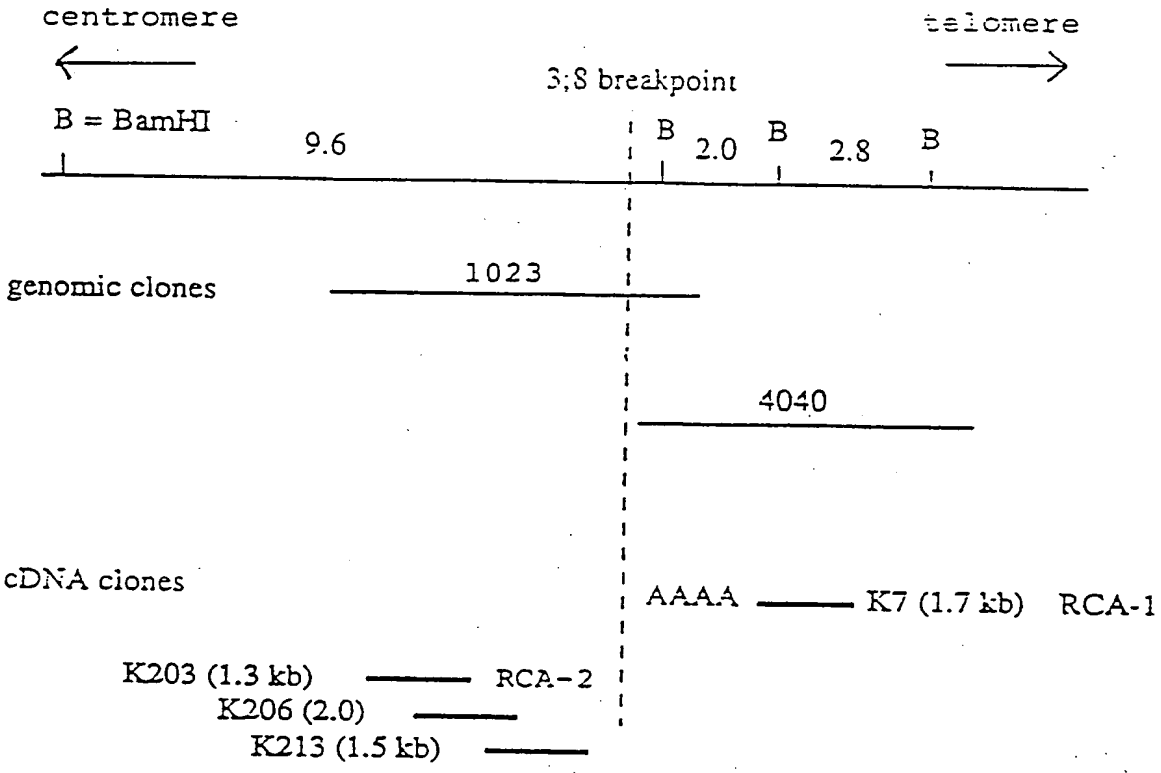


FIG. 12A

FIG. 12B

FIG. 12C


centromere
telomere
3;8 breakpoint
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B
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2.8
B
genomic clones
1023
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cDNA clones
AAAA
K7 (1.7 kb)
RCA-1
K203 (1.3 kb)
RCA-2
K206 (2.0)
K213 (1.5 kb)

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10229

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68; C07H 17/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
search terms: renal carcinoma associated gene, chromosome3, tumor, carcinoma

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NUCLEIC ACIDS RESEARCH, Volume 17, Number 16, issued 1989, Sommer et al., "Minimal homology requirements for PCR primers", page 6749; see entire document.	1-10, 12, 14, 16, and 17
X	NATURE, Volume 329, Number 6138, issued 01 October 1987, Naylor et al., "Loss of heterozygosity of chromosome 3p markers in small-cell lung cancer", pages 451-454; see: (i) page 451 (abstract); (ii) page 452, left column, second paragraph, and right column, second and third paragraphs; and (iii) page 453, left column first paragraph, and right column, second and third paragraphs.	1-17

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 OCTOBER 1994	Date of mailing of the international search report 09 JAN 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BRADLEY L. SISSON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

In. ..national application No.
PCT/US94/10229

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ONCOGENE, Volume 8, Number 2, issued February 1993, Yamakawa et al., "Frequent homozygous deletions in lung cancer cell lines detected by a DNA marker located at 3p21.3-p22", pages 327-330; see pages 327-329.	1-17
X	INTERNATIONAL JOURNAL OF CANCER, Volume 53, Number 3, issued February 1993, Van Der Hout et al., "Loss of heterozygosity at the short arm of chromosome 3 in renal-cell cancer correlates with the cytological tumor type", pages 353-357; see pages 353-356.	1-17
A	GENOMICS, Volume 10, Number 8, issued July 1991, Mendez et al., "Rapid screening of a YAC Library by Pulsed-Field Gel Southern Blot Analysis of Pooled YAC Clones", pages 661-665; see pages 661-664.	1-17
X	CANCER RESEARCH, Volume 51, Number 3, issued 01 February 1991, Ogawa et al., "Allelic Loss at Chromosome 3p Characterizes Clear Cell Phenotypes of Renal Cell Carcinoma", pages 949-953; see pages 949-951.	1-17
X	THE NEW ENGLAND JOURNAL OF MEDICINE, Volume 301, Number 11, issued 13 September 1979, Cohen et al., "Hereditary Renal-Cell Carcinoma Associated with a Chromosomal Translocation", pages 592-595; see entire document.	1-17
A	SCIENCE, Volume 236, Number 4803, issued 15 May 1987, Burke et al., "Cloning of Large Segments of Exogenous DNA into Yeast by Means of Artificial Chromosome Vectors", pages 806-812; see pages 806-811.	1-17
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, U. S. A., Volume 82, Number 20, issued October 1985, Drabkin et al., "Translocation of c-myc in the hereditary renal cell carcinoma associated with a t(3;8)(p14.2;q24.13) chromosomal translocation", pages 6980-6984; see pages 6980-6983.	1-17
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, U. S. A., Volume 85, issued March 1988, Kovacs et al., "Consistent chromosome 3p deletion and loss of heterozygosity in renal cell carcinoma", pages 1571-1575; see pages 1517-1574.	1-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10229

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6; 536/23.5, 24.31

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

435/6, 91.1, 91.2, 270; 536/23.1, 23.5, 24.31, 25.3; 935/77, 78

C12P 19/34; C12N 1/08; C07H 17/00, 23/00